



Risk factors causing postweaning multisystemic wasting syndrome (PMWS) onset in Swiss pig farms

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Abstract: Postweaning multisystemic wasting syndrome (PMWS) was enzootic between 2003 and 2008 in Switzerland. Nevertheless, infectious risk factors including porcine reproductive and respiratory syndrome virus (PRRSV) were missing at all or were seen only sporadically (enzootic pneumonia and actinobacteriosis). In a case-control study, 30 farms with PMWS affected pigs were compared to 30 inconspicuous farms ("matched pairs"). The case-control allocation was verified by PCV2 DNA measurements of 5 healthy weaned pigs in each control farm, 5 healthy and 5 PMWS affected weaners in each PMWS affected farm. Diseased pigs showed in average 1.8×10^8 DNA templates per ml serum significantly higher than healthy pigs from control farms with 1×10^6 DNA templates per ml serum. Virus load in healthy pigs did not differ between control- and PMWS affected farms. PMWS mainly emerged among affected pigs in the 5th to 8th week of age. In a logistic regression model risk factors were identified such as high occupancy in weaning pens ($p = 0.002$), large groups in gestation facilities ($p = 0.03$) as well as reduced birth weight < 1.3 kg ($p = 0.04$). We suggest these factors might have led to chronic stress e.g. through influencing negatively social interaction in pigs or disturbances of the maturing immune system. Heavy fly and rodent infestation might not only be viewed as a vector for disease transmission, but, also as a stress factor. In den Jahren 2003 – 2008 entwickelte sich das «Postweaning Multisystemic Wasting Syndrome» (PMWS) in der Schweiz zu einer Epizootie, obwohl beschriebene infektiöse Risikofaktoren für das Angehen von PMWS, wie das «Porcine Reproductive und Respiratory Syndrome Virus» (PRRSV) fehlen oder bei der enzootischen Pneumonie (EP) und der Aktinobazilliose eine untergeordnete Rolle spielen. In einer Fall-Kontroll-Studie wurden 30 PMWS-Problembetriebe und 30 Kontrollbetriebe («matched pairs») auf Risikofaktoren analysiert. Kranke Schweine wiesen mit durchschnittlich 1.8×10^8 DNA-Kopien/ml Serum signifikant höhere Virustiter auf als gesunde Schweine aus den Kontrollbetrieben, welche 1×10^6 DNA-Kopien/ml Serum aufwiesen. Der Virustiter von gesunden Tieren unterschied sich nicht signifikant zwischen PMWS- und Kontrollbetrieben. PMWS trat meist zwischen der 5.–8. Lebenswoche auf. In einer logistischen Regressionsanalyse wurden Risikofaktoren wie hohe Belegdichten in den Absetzbuchten ($p = 0.002$), grosse Galtssauengruppen ($p = 0.03$) sowie ein vermindertes Geburtsgewicht < 1.3 kg ($p = 0.04$) identifiziert. Diese Faktoren können zu chronischem Stress führen, welche die soziale Interaktion stören und die Ontogenese des fetalen Immunsystems negativ beeinflussen. Auch Fliegen- und Schädnerbefall sind nicht zu unterschätzende Risikofaktoren beziehungsweise Stressfaktoren.

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Risk factors causing postweaning multisystemic wasting syndrome (PMWS) onset in Swiss pig farms

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Summary

Postweaning multisystemic wasting syndrome (PMWS) was epizootic between 2003 and 2008 in Switzerland. Nevertheless, infectious risk factors including porcine reproductive and respiratory syndrome virus (PRRSV) were missing at all or were seen only sporadically (enzootic pneumonia and actinobazillosis). In a case-control study, 30 farms with PMWS affected pigs were compared to 30 inconspicuous farms ("matched pairs"). The case-control allocation was verified by PCV2 DNA measurements of 5 healthy weaned pigs in each control farm, 5 healthy and 5 PMWS affected weaners in each PMWS affected farm. Diseased pigs showed in average 1.8×10^8 DNA templates per ml serum significantly higher than healthy pigs from control farms with 1×10^6 DNA templates per ml serum. Virus load in healthy pigs did not differ between control- and PMWS affected farms. PMWS mainly emerged among affected pigs in the 5th to 8th week of age. In a logistic regression model risk factors were identified such as high occupancy in weaning pens ($p=0.002$), large groups in gestation facilities ($p=0.03$) as well as reduced birth weight <1.3 kg ($p=0.04$). We suggest these factors might have lead to chronic stress e.g. through influencing negatively social interaction in pigs or disturbances of the maturing immune system. Heavy fly and rodent infestation might not only be viewed as a vector for disease transmission, but, also as a stress factor.

Keywords: PMWS, PCV2, risk factors, problem farms, control farms

Risikofaktoren für das Angehen des Postweaning Multisystemic Wasting Syndrom (PMWS) in Schweizer Schweinezuchtbetrieben

Zusammenfassung

In den Jahren 2003-2008 entwickelte sich das „Postweaning Multisystemic Wasting Syndrom“ (PMWS) in der Schweiz zu einer Epizootie, obwohl beschriebene infektiöse Risikofaktoren für das Angehen von PMWS, wie das „Porcine Reproductive und Respiratory Syndrome Virus“ (PRRSV) fehlen oder bei der enzootischen Pneumonie (EP) und der Aktinobazillose eine untergeordnete Rolle spielen. In einer Fall-Kontroll-Studie wurden 30 PMWS-Problembetriebe und 30 Kontrollbetriebe („matched pairs“) auf Risikofaktoren analysiert. Kranke Schweine wiesen mit durchschnittlich 1.8×10^8 DNA-Kopien/ml Serum signifikant höhere Virustiter auf als gesunde Schweine aus den Kontrollbetrieben, welche 1×10^6 DNA-Kopien/ml Serum aufwiesen. Der Virustiter von gesunden Tieren unterschied sich nicht signifikant zwischen PMWS- und Kontrollbetrieben. PMWS trat meist zwischen der 5.-8. Lebenswoche auf. In einer logistischen Regressionsanalyse wurden Risikofaktoren wie hohe Belegdichten in den Absetzbuchten ($p=0.002$), grosse Galtsauengruppen ($p=0.03$) sowie ein vermindertes Geburtsgewicht <1.3 kg ($p=0.04$) identifiziert. Diese Faktoren können zu chronischem Stress führen, welche die soziale Interaktion stören und die Ontogenese des fetalen Immunsystems negativ beeinflussen. Auch Fliegen- und Schädigernbefall sind nicht zu unterschätzende Risikofaktoren beziehungsweise Stressfaktoren.

Schlüsselwörter: PMWS, PCV2, Risikofaktoren, PMWS-Betriebe, Kontrollbetriebe

Introduction

Postweaning multisystemic wasting syndrome (PMWS) was first observed 1991 in Western Canada and from 1996 on recognized as a pig specific disease (Harding and Clark, 1997b). The disease is found in any pig producing country (Patterson and Opriessnig, 2010) and is mainly caused by porcine circovirus type 2 (PCV2) (Segales et al., 1997; Allan et al., 1998). Pigs develop disease usually between 4-14 weeks of age (Harding et al., 1998; Allan and Ellis, 2000a; Rodríguez-Arriola et al., 2002). In some instances, PMWS was also observed in pigs 30 weeks of age (Pallares et al., 2002). Clinical manifestations of PMWS include wasting, profuse and untreatable

diarrhea, respiratory distress and less often anemia and icterus (Harding and Clark, 1997b). Morbidity varies between 4-30% (Nielsen et al., 2008), and may even increase exceptionally to 50-60% (Segales und Domingo, 2002). Lethality is in average between 70-80% and reaches sometimes 100% (Cheung et al., 2007). Thus, the financial losses are high and estimated in Europe of 562 to 900 Millions € per annum (Armstrong and Bishop, 2004).

PCV2-DNA amplicate can be easily detected in lymphatic organs as well as in nose fluid, feces, urine and serum independently of pig's health status (Allan and Ellis, 2000a; Harding, 2004). Nevertheless, diseased pigs have higher virus titers than healthy animals (Olvera et al., 2004; Sibila et al., 2004; Segales et al., 2005b; Fort et al., 2007). Pigs with viral genomes $<10^6$ templates/ml serum are diagnosed PMWS negative, while viral genome values between 10^6 - 10^7 per ml serum are questionable, however pigs with PCV2 genomes $>10^8$ per ml serum are PMWS diseased (Liu et al., 2000; Brunborg et al., 2004).

In many pig infection experiments, PMWS was initiated in PCV2 infected animals solely with a co-infection by porcine parvovirus (PPV) (Allan et al., 1999a; Krakowka et al., 2000; Kim et al., 2003a), PRRSV (Allan et al., 2000c; Rovira et al., 2002) or *M. hyopneumoniae* (Opriessnig et al., 2004).

Noninfectious, management dependent risk factors include non hygienic husbandry, inadequate quarantine- and biosafety measures (De Jong et al., 2003; Madec et al., 2000; 2008), insufficient colostrum supply (Corr  g   et al., 2001; Madec et al., 2008), high pig density in pens and barns and mixing of pig groups (Albina et al., 2001; Rose et al., 2003; Rathkjen and Riising, 2004). Pigs have a higher risk to develop PMWS when weaned earlier (Lopez-Soria et al., 2005). Male piglets, piglets with a low birth weight or light weight weaners develop PMWS significantly more often than female piglets, piglets with higher birth weight or heavy weaners (Corr  g   et al., 2001).

Multiple investigations revealed pig breeds with higher susceptibility to PMWS development (Lopez-Soria et al., 2005; Sibila et al., 2005; Opriessnig et al., 2006). Infected boars may shed PCV2 irregularly over weeks (Larochelle et al., 2000). Additionally, the risk of PMWS transmission with PCV2 contaminated sperm is controversial. Thus, Larochelle et al., (2000) and Mateusen et al., (2004) assume the risk as small, while Kim et al., (2003b) and Schmoll et al., (2003) estimate the transmission risk as high.

PMWS was first described 2001 in Switzerland (Borel et al., 2001). Nevertheless, PCV2 infections were dated in retrospective studies back to the year 1986 by immunohistochemistry (IHC) (Staebler et al., 2005) and by PCR even to 1977 (Wiederkehr et al., 2009). PMWS was more often diagnosed since the end of 2003 in pig dense regions. The disease dissemination followed trade ways throughout Switzerland (Welti et al., 2009). To this day it could not be determined with surety what infectious or non-infectious factors in addition to PCV2, mutations of the viral genome or a combination thereof might be responsible for the Swiss epizootic.

The Swiss PMWS disease increase was more surprising as many of the implied infectious risk factors including PRRS were not present or only sporadically appeared due to enzootic pneumonia (EP) and actinobazillosis, eradicated from 1996 to 2004. Switzerland is free of any diseases listed by the "Office International des Epizooties (OIE)". PRRSV absence is documented in Switzerland (Corbellini et al., 2006; Schwermer und Sievi, 2010). Respiratory diseases, enzootic pneumonia (EP) and actinobazillosis are „zu bekämpfende Tierseuchen“, since 1995 in Switzerland. EP, APP and PRRS immunization are prohibited by law. Thus, an immune system overstimulation of the piglet described by other authors as risk factor seems less likely (Allan et al., 2001; Kyriakis et al., 2002; Opriessnig et al., 2003). Also, no obvious changes occurred over the past years in husbandry, feeding, pig genetic and Swiss pig management prior to the PMWS epizootic. Swiss pig farms with an average of 34 sows or 118 fattening pigs are smaller than the pig farms in the rest of Europe (Data from SUISAG business unit SGD® (2007)).

It is also suggested that worldwide transport with infected animals played a central role to PCV2 transmission (Firth et al., 2009). However, this risk factor had negligibly contributed to the Swiss PMWS epizootic since the law prohibits livestock transport through Switzerland and only a few breeding pigs are imported; animal trafficking is further reduced by strict customs import requirements.

The goal of this investigation was to identify risk factors in PMWS farms with the help of a case control study.

Material and methods

Farm selection criteria

30 PMWS-farms were chosen with the help of databases from SGD[®] and Institute of Veterinary Pathology (University of Zurich) that also compiled pig data from 2005-2008. A farm was defined PMWS diseased based on the following criteria: (i) for the single pig according to Sorden et al. (2000), and, (ii) for the definition of the farm status by the 6th Frame work and the American Association of Swine Veterinarians (<http://www.aasp.org/aasv/position-PCVAD.htm>, 4. February. 2007).

Each PMWS farm was compared to a control farm in close proximity and with similar animal occupancy (matched pairs). The pig producers were first informed about the project and later invited to participate. During a farm inspection, a questionnaire was completed to generate the farm's PMWS epizootic profile. It was used to compare match pair control-farm characteristics. The barns' dimensions were calculated either using a blueprint or directly measured with the help of a laser power meter. A door that could be locked and separate air volume defined a room.

Defining PCV2 DNA concentration in pig serum

Farm allocation was controlled by examining 5 wasting and 5 healthy weaners from a PMWS affected farm and 5 aged matched from a healthy farm. Virus concentration was measured from blood sample by sybr green based quantitative PCR (qPCR) (manuscript in preparation).

Statistics

Questionary data were filed and analyzed with the software, FileMakerPro 7. StatView 5.1 software was used for mono- and mutivariate analysis. Continuous values were evaluated by the T-test and categorical values by the Chi-square-test. Values $p \leq 0.05$ were evaluated as significant and values $0.05 > p < 0.2$ as tendency. Parameters were used for the "full model" that were either significant or showed a tendency in the monovariate analysis. A logistic regression was applied to reverse calculations (Altman, 2006). In the final model, parameters were chosen that contained $p \leq 0.05$ in the mutivariate model.

Results

This case-control study was performed with 30 PMWS and 30 control farms (matched pairs) before PCV2 vaccination introduction.

Sera from wasting weaners had 1.8×10^8 PCV2 DNA templates /ml serum. These values were significantly higher ($p=0.0003$) than the average of 1×10^6 PCV2 DNA/ml serum found in age matched weaners from the control farms. The virus content in the healthy pig sera from control and PMWS farms was not significantly different.

Parameter comparison between PMWS and control farms

No significant differences were noticed between PMWS diseased and control farms in pig breed nor herd characteristics (total depopulation-repopulation, partial depopulation-repopulation) or herd's replacements. We also found no significant difference between control and PMWS farms in the purchase of gilts or the use of farm owned boar's natural services or artificial insemination. Other parameters such as MMA-prevalence (Metritis Mastitis Agalactia), weaning, cleaning and disinfection of dams before moving to the farrowing pens were also not significantly different between PMWS and control farms. However, we did not further examine cleaning and disinfection qualities.

Area and room volume

We investigated all farms including farrowing facilities and nurseries for area, volume, partitions and pen size of the different barns.

For farrowing facilities, areas per pig ($p=0.0072$) as well as volume per pig ($p=0.0115$) were significantly smaller in the PMWS diseased farms than in the matched pair control farms (Table 1). Also, area ($p=0.0593$) and volume ($p=0.0687$) of gestation facilities from PMWS diseased farms tend to be smaller than their control counterpart (Table 1). Additionally, pen partitioning in farrowing facilities was by trend less ($p=0.0810$) and in gestation facilities significantly less ($p=0.0084$) on PMWS farms (Table 1). Farrowing facilities that were smaller by area ($p=0.0382$) tended also to be smaller in volume ($p=0.1848$) and contained in average less pens that were bigger ($p=0.1041$) than their matched pair control barns (Table 1). These led to crowding ($p=0.004$) and consequently to diminished piglet space ($p=0.1830$) compared to control farms (Table 1).

Rodent infestations

We found that farrowing facilities contained significant ($p=0.0654$) rodent infestations and nurseries with tendency ($p=0.1503$) to rodent infestations in PMWS farms when

compared to control farms (Table 1), although these observations were not completely confirmed by the farmers' own statements.

Flystrike and fly control

According to owner statements flies were significantly more abundant in gestation facilities ($p=0.0048$) and tended to be problematic in farrowing facilities ($p=0.1469$) on PMWS farms in comparison to control matched pair. This was supported by the fact that more fly controls were used on PMWS affected farms than on the matched pair controls, i.e., fly controls used significantly more often in nurseries ($p=0.0350$) and by tendency in farrowing facilities ($p=0.1503$) in PMWS farms. Nevertheless, fly control method and efficacy were not compared.

Birth weight and birth control

Birth weight was significantly smaller than 1.3 kg ($p=0.0098$) on PMWS affected farms compared to control farms according to farmers' surveys. Noticeably, the farmers used birth control ($p=0.1100$) in PMWS problematic farms more than in the control farms. Interestingly, compositions of piglet creep nor time or manner of iron supplementation (oral or parental) were statistically different between PMWS affected and control farms.

Antibiotic use

Antibiotics were more frequently used ($p=0.0092$) in PMWS diseased farms than in their counterpart control. Out of 30 PMWS problematic farms, 16 regularly used tetracycline or tylosin as mono-substance or in combination with chlortetracycline-sulfadimidine-tylosin. Only 3 control farms commonly used antibiotics.

Multivariate data analysis

After multivariate logistic regression with step back procedure on values $p<0.2$ for the total model and $p<0.05$ for parameters in the final model we found 3 parameters significantly different between PMWS problematic farms and their controls: i) occupancy in nurseries ($p=0.002$), ii) group size in gestation facilities ($p=0.03$) as well as iii) piglets <1.3 kg birth weight ($p=0.04$).

Discussion

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239 Although PCV2 is the main agent, there may be several other additional factors
240 involved in PMWS initiation (Segales et al., 1997; Allan et al., 1999b). Generally
241 infections with PCV2 are immune suppressive (Darwich et al., 2004; Segales et al.,
242 2004a) which may lead to secondary infections (Segales et al., 2004b). In many
243 experiments PMWS could only be induced with additional PPV infection (Allan et al.,
244 1999a; Krakowka et al., 2000; Kim et al., 2003a) or PRRSV infection (Allan et al.,
245 2000c; Rovira et al., 2002) or with *M. hyopneumoniae* infection (Opriessnig et al.,
246 2004). The obvious increase in use of antibiotics to combat secondary bacterial
247 infection in PMWS problematic was already described by other authors (Madec et al.,
248 2000), which did not improve the diseased farms fate. No obvious new infectious
249 agents, changes in pig genetic or management occurred before initiation of the Swiss
250 PMWS epizootic. Thus, a virus genetic shift was suggested (Wiederkehr et al. 2009).
251 However, farm specific factors might also simply influence disease course including
252 high pig density and mixing of pig groups (Albina et al., 2001; Rose et al., 2003;
253 Rathkjen und Riising, 2004) or colostrum undersupply (Corrége et al., 2001; Madec
254 et al., 2008).

255 Indeed in our case-control study, pig density turned out to be a risk factor for
256 development of PMWS. In the PMWS farms the area and volume was smaller in the
257 nurseries and pen partitioning were fewer than in the control farms. Thus, in the
258 PMWS farms weaner groups were larger and individual weaner had less space.
259 Additionally on many farms, weaner places are limited in numbers and an “all in all
260 out” is hardly manageable as no auxiliary pens are available. Growth retarded
261 weaners are generally sorted and mixed together in a smaller pen. Since PMWS
262 diseased pigs grow slower, contain higher blood virus content and shed higher PCV2
263 (Segales et al., 2005b; Fort et al., 2007), it adds infection pressure in an already
264 crowded pen. Our wasting pigs contained about 180 times higher PCV2
265 concentrations in serum than healthy pigs. Of note, stress caused by changing pens
266 or mixing of groups down regulates killer cell activity Sutherland et al., (2006)
267 especially in socially lower pigs.

268 According to the farmer's survey, most pigs contracted PMWS at the age of 6 to 8
269 weeks of age. To our surprise, in this study few possible risk factors could be
270 excluded e.g. i) a more vigorous birth control, ii) pre-established and generous lair for

suckling piglets or iii) obligate MMA surveillance program. However, a standardized MMA procedure is missing and a follow up is needed.

We found reminiscent to others that birth weight had a significant influence on the piglets developing (Corr  g   et al., 2001). Farmers from PMWS farms indicated that piglets were born commonly with a birth weight smaller than 1.3 kg. It may be speculated that smaller or weaker piglets particularly in larger litters are pushed off the sow's udders causing them to catch less colostrum and thus less amounts of maternal antibodies. Hence, blood of dominant piglets have significant higher antibody levels and better phagocytosis activity than socially minor piglets (Sutherland et al., 2006).

We noticed that gestations facilities were fewer in numbers in PMWS diseased farms and this caused overall bigger group sizes. The problem is further exasperated as pregnant dam groups are hardly kept constant. Parturient dams are moved to the farrowing facilities and serviced dams are newly introduced into the pregnant sow group depending on the production rhythm, which inadvertently causes tension and rank fights. Chronic stress among pregnant dams may interfere with fetal immune system ontogeny and may negatively effect fetuses' humoral and cellular immune responses (Tuchscherer et al., 2002).

PCV2 is extremely resistant to chemical and heat treatment (Welch et al., 2006). It is possible that PCV2 may be transmitted by a live vector. During an investigation of two pig farms, 65% of dead mice and 24% of dead rats turned out to be PCV2 infected while mice or rats not close to any PMWS infected farms were not infected (Lorincz et al., 2010). We also found that rodents and flies tended to be a bigger problem needing more intense combatting in the PMWS farms compared to the control farms. Thus we suppose that rodents as well as flies may be important vectors for transmission of the disease. Flies may also be regarded a chronic "stressor" to afflicted animals in addition to being a vector to transmission of the disease.

Our studies revealed significant risk factors including fewer places in nurseries, fewer gestation facilities and, low birth weight that generally disturbs social interaction among the animals and in particular, stress that may affect the maturing immune system. A variety of negative influences including both, infectious or non-infectious causes on the immune system of the piglet and dams seem to interplay. We assume the genetic shift of PCV2 genotypes (Wiederkehr et al., 2009) to more pathogenic

virus variants as well as the risk factors identified here which were considered as chronic stress factors, may act in concert leading to the break-down of the animals defense system and development of post weaning multisystemic wasting syndrome.

Table 1: Tendency ($p \leq 0.2$) und significant ($p \leq 0.05$) parameters listed in the monovariate and ($p \leq 0.05$) final model evaluation.

		PMWS affected farms	Control farms	p-values monovariate	p-values final model
Farrowing facilities					
Area [m^2]	a	73.5 (averages)	112.1 (averages)	0.0072	
Volume [m^3]	a	175	275	0.0115	
Numbers of pens	a	8.9	11.5	0.0810	
Gestations facilities					
Area [m^2]	a	107	186	0.0593	
Volume [m^3]	a	265	613	0.0687	
Numbers of pens	a	4.6	8	0.0084	0.0320
Nurseries					
Area [m^2]	a	73.4	103.6	0.0382	
Volume [m^3]	a	182.7	255.4	0.1848	
Pen area [m^2]	a	17.4	14.9	0.1041	
Pig per pen	a	45.6	34.5	0.0040	0.0020
Area per weaner [m^2]	a	0.38	0.43	0.1830	
Rodents infestations					
Farrowing facilities yes/no	b	5 / 25 (Numbers of farms)	0 / 30 (Numbers of farms)	0.0654	
Nursery yes /no	b	2 / 28	0 / 30	0.1503	
Flystrike					
Gestation facilities yes/ no	c	14 / 16	4 / 26	0.0480	
Nursery yes/no	c	14 / 16	7 / 23	0.1469	
Fly control					
Nursery yes/no	c	22 / 8	14 / 16	0.0350	
Farrowing facilities yes/no	c	22 / 8	15 / 15	0.1503	
Birth weight					
<1.3kg / >1.3 kg	c	10 / 20	2 / 28	0.0098	0.0415
Birth control					
always/ sometimes/ never	c	11 / 13 / 6	5 / 23 / 2	0.1100	

a) Measured or calculated parameters, b) our own observations, c) farmers statements.

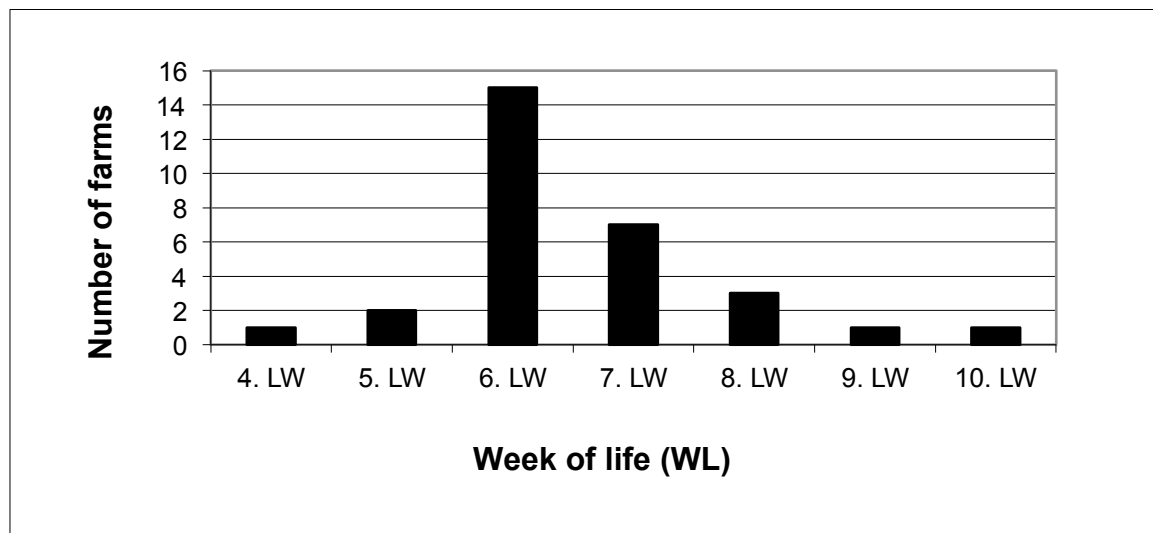


Figure 1: PMWS occurrence in weeks of pig life according to farmers' statements.